

Etiology of Mastitis in Herds of Dairy Cows and Ewes Situated in Marginal Parts of Slovakia

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Abstract

A significant proportion of agricultural production in Slovakia is situated in areas that did not have natural conditions to this production. On the base of soil, and ecological conditions are often these areas are called marginal. Breeding of ruminants with market milk production represents a significant proportion of livestock production in marginal regions. Mastitis is one of the biggest problems of dairy producers in these regions causes great losses in the livestock economy. The aim of this study was evaluated occurrence and etiology of mastitis in two dairy herds of cows and ewes situated in marginal parts of Slovakia. The diagnosis of mastitis was performed on the basis of clinical examination of the udder, macroscopic evaluation of milk, with the evaluation of Californian mastitis test (CMT) and bacteriological analysis of individual raw milk samples. From total 1193 and 914 quarter cow's milk samples were 17.3% and 12.1% positive to CMT, respectively. In monitored sheep herds were positive 10.0% and 23.4% halves milk samples to CMT, respectively. The prevalence of intramammary infection (IMI) in the monitored herds of cows was 14.6% to 10.5%, respectively. In dairy herds of ewes was prevalence of mastitis 8.5% to 16.7%, respectively. In all monitored cows and sheep herds were confirmed predominantly subclinical forms of IMI. The highest percentage of etiological agents in all monitored herds had coagulase negative staphylococci (CNS) and coagulase positive staphylococci (CPS) especially *Staphylococcus aureus*. Except for staphylococci were *Streptococcus uberis* and *Streptococcus spp.* most frequently pathogens isolated from dairy cows. Given the variety of factors causing IMI will milk production and economic prosperity of holdings located in marginal areas depend primarily on the expertise and skills to implement preventive anti-mastitis methods, and technological systems to own agricultural production.

Keywords: Marginal Regions; Slovakia, Cows; Ewes; Mastitis; Bacterial Pathogens

Introduction

Marginal regions are part of the area Slovakia which in terms of the economy breeding ruminants can efficiently produce animal commodities only occasionally. Geographic, social and economic stability of these regions is strongly influenced by breeding of ruminants with market milk production. The breeding of sheep and dairy cows accounts for up to 75% of the production of these areas [1-3].

Products from dairy cows and sheep are unique, especially in the field of rational nutrition of consumers. Many of these products and specialties can be included among the functional foods [4,5].

Udder health affects the yield and quantity of milk obtained from the cows and ewes is currently the main breeding aim of many agricultural farms localized in marginal regions and play a crucial role in the economy of cooperatives and farms. EU legislation emphasizes that milk intended for consumption must come exclusively from healthy animals [5,6].

Dairy animals are exposed to numerous genetic, physiological, and environmental factors that can compromise host immunity and increase the incidence of mastitis. Emphasis on genetic selection to maximize milk production has increased metabolic stresses associated with milk synthesis and secretion, and a negative correlation exists between milk production capacity and resistance to mastitis [7-9].

Mastitis, an inflammatory response of the mammary gland caused usually by bacteria, is probably the most costly of the infectious, endemic diseases to affect dairy cows and other dairy species. Losses caused by mastitis include reduced milk yield and quality, costs of veterinary care, discarded milk and shortening of productive life [10,11].

The disease is characterized by several physical and chemical alterations of the milk and corresponding pathological changes in the mammary tissue depending on the type of the disease. The disease is usually local but may become systemic, although rarely, in immunocompromised animals [12,13].

Mastitis is caused by interaction of various factors associated with the animal, pathogens and the environment, so nature and duration of the disease varies accordingly. More than 140 different microorganisms are recognized to cause mastitis. Infectious agents like bacteria, viruses, fungi and algae are mostly the primary causes of the disease. Bacteria are the most common cause of mastitis and hence bacteriological culture is routinely used in the laboratory diagnosis [14,15].

Authors Vasil, *et al.* [16] and Tenhagen, *et al.* [17] indicate that up to 95% intramammary infection (IMI) is caused by bacterial pathogens (Figure 1).

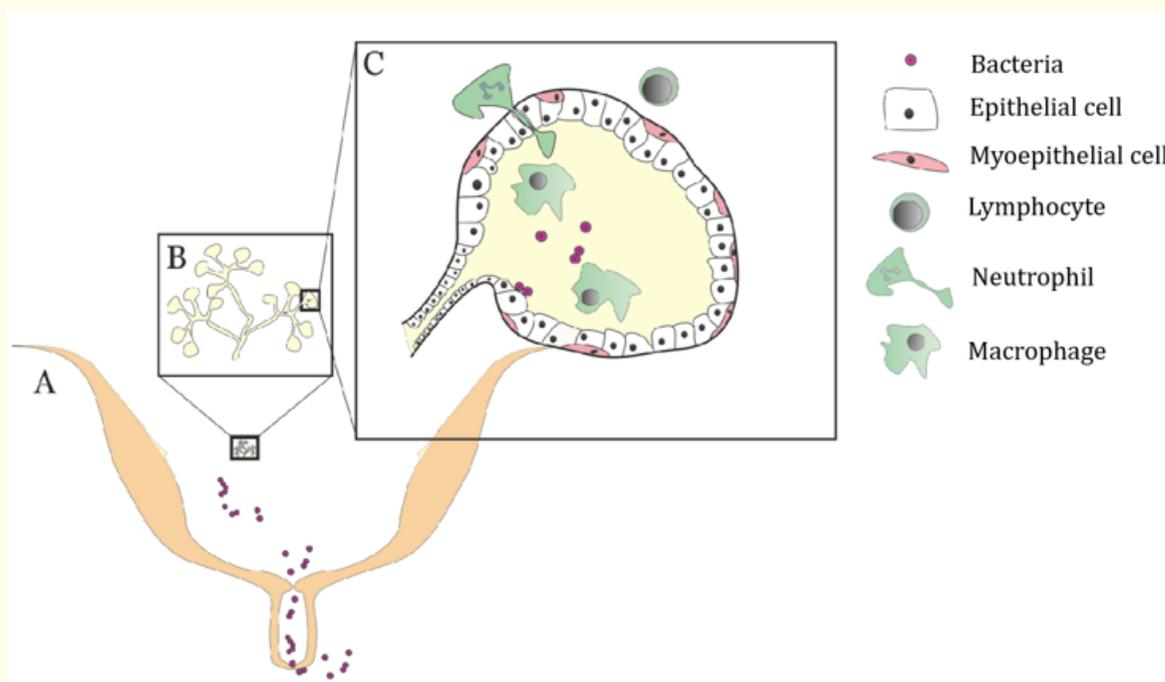


Figure 1: Schematic representation of the bovine mammary gland.
 Note: (A) gland parenchyma with secretory units and milk ducts (B), and alveolus (C) of the udder with bacteria and immune cells [18].

Globally, CNS and *S. aureus* are the most common mastitis causing pathogens in cows, buffaloes, ewes, does, llamas, dromedaries, rabbits, dolphins and women. They are closely followed by streptococci and *E. coli*, which in some species or settings may have a similar or higher prevalence than that of staphylococcal mastitis. Less commonly, other Gram-positive (*Actinomyces spp.*, *Corynebacterium spp.*, *Bacillus spp.*, *Mycobacterium spp.*, *Enterococcus spp.*, *Clostridium spp.*) and Gram-negative (*Klebsiella spp.*, *Enterobacter spp.*, *Citrobacter spp.*, *Serratia spp.*, *Proteus spp.*, *Pasteurella spp.*, *Mannheimia haemolytica*) bacteria and mycoplasma may be involved in the etiology of mastitis while cases due to molds or yeasts are rare [9].

In dairy animals, mastitis causing pathogens are classified depending on their epidemiological behavior in contagious and environmental [9,19]. Among contagious pathogens belong *S. aureus* [20] *Streptococcus agalactiae*, and *Mycoplasma spp.* are included [10,21,22].

The main reservoir for contagious pathogens is the mammary gland; consequently, these bacteria are spread from cow to cow or between quarters/halves of the same animal during the milking process. In contrast, environmental pathogen reservoirs include bedding, pasture, and other unanimated objects. These bacteria are isolated from the skin, teat ends, and teat canals where they can gain access to the gland causing IMI. *Streptococcus* other than *agalactiae*, *Enterococcus spp.*, CNS, and coliform bacteria including *Escherichia coli* and *Klebsiella spp.* are included in this group [3,9].

Purpose of the Study

The quality and quantity of milk obtained from the cows is important for the dairy sector. Mastitis is considered the most frequent and most costly production disease in dairy herds localized in marginal regions. The aim of this study was evaluated occurrence and etiology of mastitis in two dairy herds of cows and ewes situated in marginal parts of Slovakia.

Materials and Methods

Monitored herds

The practical part of the study was performed on two cows and sheep dairy herds situated in marginal parts of Slovakia (Kysuce, Zemplin), kept under standard animal husbandry and hygiene conditions.

Cows and milking

Herds size ranged from 300 (A) to 230 (B) dairy cows of Holstein and Slovak Pied cattle breed between 2nd - 4th lactation were used, respectively. Dairy cows from both farms were kept in a free housing system with a separate calving barn and equipped with individual boxes with bedding and were allowed *ad libitum* access to water. All herds were fed total mixed ration based on grass silage, maize silage and concentrate according to international standards [23]. All cows were milked twice daily. The cows from herd A were milked in parallel parlor Boumatic 2 x 12 Xpressway (Wisconsin, USA). In fishing-milking parlour (FarmTec) 2 x 10 were milked cows from herd B. Blanket dry cow therapy was implemented in both herds.

Sheep and milking

The herd A (240 sheep) consisted of the breeds, Improved Valaska and Lacaune. The herd B (112 sheep) consisted of the breeds, Improved Valaska and Tsigai. The ewes were milked twice a day after weaning of their lambs at the beginning of April. In herd A, machine milking was performed using two-line milk parlour 2 x 15 Miele Melktechnik, (Hochreiter Landtechnik, Germany) and in herd B sheep were milked in two-line milk parlour 2 x 12 Alfa Laval Agri (Alfa Laval, Sweden).

Examination of health status

The examination of health status included clinical examination of the mammary gland, examination fore-strip of milk, with CMT reaction, subsequent collecting of milk samples for bacteriological examination, subsequent cultivation and identification of pathogenic bacteria. Clinical examination consisted of heart rate, body temperature, respiratory rate, udder health including the mammary gland palpation, evaluation of macroscopic changes in milk, and evaluation of somatic cell count in milk using the Californian mastitis tes (CMT). CMT (Indirect diagnostic test, Krause, Denmark) was performed from the 300 and 230 milked cows on 1193 and 914 quarter milk samples, respectively. The same evaluation of CMT score were from the 240 and 112 milked ewes on 477 and 222 halves, respectively.

Milk (2 ml) from each mammary quarter or half was mixed with a CMT reagent. After homogenisation, the samples were classified according to the degree of viscosity. CMT scores were classified in four scores: 0 = negative or trace (T), 1 = weak positive, 2 = distinct positive and 3 = strong positive (Table 1).

Herds	Cows		Ewes	
	A	B	A	B
	%	%	%	%
Healthy quarters/halves	82.7	87.9	90.0 ^a	76.6 ^b
CMT positive quarters/halves ¹	17.3	12.1	10.0 ^a	23.4 ^b
Infected quarters/halves ²	14.6	10.5	8.5 ^a	16.7 ^b
Number of examined quarters/halves (n)	1193	914	477	222
Number of animals in herd (n)	300	230	240	112

Table 1: Examination of herds in dairy cows and sheep.

Note: ¹: Evaluation of CMT test with score 1 - 3; ²: Evaluation on the base of CMT test and positive microbiological cultivation of milk samples.

^{a,b}: Different superscript letters among herds of cows and ewes in same row means $P < 0.05$.

Quarter cow's milk samples and halves sheep's milk samples of the secretion (10 ml) were then collected with aseptic techniques in accordance with National Mastitis Council guidelines [24]. The samples were cooled and immediately transported to the laboratory.

Laboratory analyses

The milk samples were investigated for bacterial pathogens according to commonly accepted rules Malinowski, *et al* [25]. Milk samples were (10 µl) incubated at 37°C for 24 hours by primo-cultivation on blood agar base with 5% of defibrinated blood and all growing isolates were characterized by classic microbiological methods and consistent cultivation on specific cultivation media: Staphylococcal medium N° 110, Baird-Parker agar, Edwards Medium, Mac Conkey Agar (Oxoid, (OXOID Ltd., Basingstoke, Hants, UK) (Figure 2).



Figure 2: Clinical mastitis cases with positive bacteriological cultivation.

Note: (A - A1) sheep clinical IMI caused of *S. aureus*, (B - B1) cow clinical IMI caused of *Str. uberis*.

In addition to evaluation of bacterial growth characteristics another assays were used to bacterial species determination: pigment and coagulase production, catalase activity, hemolysis, Gram staining and other virulence factors. Bacteria *Staphylococcus spp.* were selected for the tube coagulase test (Staphylo PK, ImunaPharm, SR). Suspect colonies *Staphylococcus spp.*, *Streptococcus spp.* and *Enterobacteriaceae spp.* were isolated on blood agar and cultivated at 37°C for 24h and detailed identified biochemically using the STAPHY-test, STREPTO-test, resp. ENTERO-test and identification by software TNW Pro 7.0 (Erba-Lachema, CZ) with precision of detection over 90.0%.

Severity of mastitis

On the basis of clinical examination of the udder, macroscopic evaluation of milk, with the evaluation of Californian mastitis test (CMT) and bacteriological analysis of milk samples were classified different forms of mastitis. Latent mastitis are characteristic only with the presence of bacterial pathogens in samples of milk without changing its consistency and SCC. Subclinical mastitis are characteristic with positive CMT score, bacteriological cultivation, increased SCC, reduced milk yield without clinical signs. Clinical mastitis are characteristic with positive CMT score, bacteriological cultivation, high level of SCC, changing the consistency of the milk, reduced or loss of milk production with clinical signs (Figure 2).

Statistical analysis

The differences in the prevalence of mastitis among herds of cows and ewes were statistically analyzed using the Chi-square test. The level of significance was set to $P < 0.05$.

Results and Discussion

Most mastitis occurs as a low grade infection, a subclinical state, which affects 10-15% of lactating animals, increasing milk leucocyte content, reducing milk production and increasing milk bacterial content. These all contribute to reduced milk value as a food and in monetary terms. The prevalence of such infections is a significant risk to uninfected animals in the herd as many mechanisms exist to expose the animals to new infection. Most commonly these include the common lying areas in housing or at pasture, the milking machine and successive contact of different cows or teats by the milker preparing the teats for milking [26].

At present in Slovakia most of the farmers of dairy ruminants have problem with subclinical mastitis SM and CM caused by staphylococci and streptococci. According to our previous studies [2,3], the prevalence of mastitis in dairy herds situated in marginal parts of Slovakia is from 7.8% to 37%.

The prevalence or number of infected cows or ewes (quarters/halves) is an unknown to most farms. These cases show no obvious signs and can only be determined from microbiological examination of milk or measurement of milk abnormality. The most common method is evaluation of somatic cell count in milk using the CMT.

The evaluation of CMT with collection of milk sample for the detection of bacteria and SCC, are routinely use in the laboratory diagnosis. From total 1193 and 914 quarter cow's milk samples were 17.3% and 12.1% positive to CMT, respectively. In monitored sheep herds were positive 10.0% and 23.4% halves milk samples to CMT, respectively. The prevalence of intramammary infection (IMI) in the monitored herds of cows was 14.6% to 10.5%, respectively. In dairy herds of ewes was prevalence of IMI 8.5% to 16.7%, respectively (Table 1).

According to Vasil', *et al.* [4,16] staphylococci and streptococci are the main etiological agents of ruminant IMI. *Staphylococcus aureus* with CNS are the most frequent isolates from subclinical and clinical cases of IMI.

In monitored dairy cows herds from 174 (14.6%) and 96 (10.5%) infected quarter cow's milk samples were isolated CNS, followed by CPS (*S. aureus* and *S. hyicus*), streptococci and enterococci, respectively (Table 2).

Pathogens	n	Latent		Subclinical		Clinical	
		A	B	A	B	A	B
		%	%	%	%	%	%
CNS ¹	103	18.0	9.3	20.1	24.7	-	-
CPS ²	32	1.7	1.0	10.3	6.2	1.7	2.1
<i>Str. uberis</i>	17	1.7	-	6.9	-	1.1	-
<i>Streptococcus spp.</i>	34	1.1	2.1	9.1	12.4	0.6	1.0
<i>E. coli</i>	28	2.3	3.1	8.0	6.2	0.6	-
<i>Enterococcus spp.</i>	36	4.1	3.1	8.6	11.3	-	-
<i>Bacillus spp.</i>	12	-	2.1	2.3	8.2	-	-
<i>Mixed infections</i>	9	-	1.0	1.7	5.2	-	-
Total	271	29.0	21.6	67.1 ^a	75.3 ^b	4.0	3.1

Table 2: Isolated bacterial pathogens of mastitis from quarter samples of raw cows’ milk

Note: n: Number of positive samples isolated from dairy cows; CNS1: Coagulase negative staphylococci (*S. schleiferi*, *S. chromogenes*, *S. xylosus*, *S. haemolyticus* and *S. sciuri*); CPS2: Coagulase positive staphylococci (*S. aureus* and *S. intermedius*), a,b: Different superscript letters among mastitis forms of in same row means $P < 0.05$.

From ewes herds were infected 41 (8.5%) and 37 (16.7) halves milk samples with the most common bacterial pathogens caused by CNS, CPS, *Str. sanguinis* and *E. coli* (Table 3).

Pathogens	n	Latent		Subclinical		Clinical	
		A	B	A	B	A	B
		%	%	%	%	%	%
CNS ¹	16	14.6	5.4	12.3	9.8	-	-
CPS ²	14	2.7	-	17.1	8.1	-	8.1
<i>Str. sanguinis</i>	7	-	-	14.6	1.6	1.6	-
<i>Streptococcus spp.</i>	11	-	-	7.2	12.5	1.6	7.2
<i>E. coli</i>	9	4.8	2.7	8.1	8.1	-	-
<i>Enterococcus spp.</i>	7	-	8.1	-	9.8	-	-
<i>Proteus spp.</i>	6	-	5.4	-	10.8	-	-
<i>Mixed infections</i>	6	4.8	-	-	10.8	-	-
Total	76	34.4 ^a	24.3 ^b	59.3 ^a	68.2 ^b	3.2 ^a	15.3 ^b

Table 3: Isolated bacterial pathogens of mastitis from half samples of raw ewes’ milk

Note: n: Number of positive samples isolated from ewes; CNS1: Coagulase negative staphylococci (*S. chromogenes*, *S. epidermidis*, *S. xylosus*, *S. haemolyticus* and *S. sciuri*); CPS2: Coagulase positive staphylococci (*S. aureus* and *S. intermedius*), a,b: Different superscript letters among mastitis forms of ewes in same row means $P < 0.05$.

The present findings are in accordance with the findings of Kivaria and Noordhuizen [27] and Tenhagen., *et al.* [17] who isolated *Staphylococcus spp.* followed by *Streptococcus spp.* and *E. coli* from subclinical and clinical cases of IMI. In many countries, CNS have been acknowledged more and more frequently as a cause of intramammary infections in dairy cattle [10,12,28]. In the case of CNS, there has been not only an increasing prevalence of such infections but also an expanding list of species reported to be involved in the process. Results of studies from different countries and continents revealed that more than 20 CNS species have been isolated from milk samples of mastitis cows, the most common being *S. chromogenes*, *S. haemolyticus*, *S. epidermidis*, *S. simulans* and *S. xylosus* [22,28,29].

In our study the most common environmental pathogens such as *S. schleiferi*, *S. chromogenes*, *S. xylosum*, *S. haemolyticus* and *S. sciuri* have been isolated from infected milk samples of cows and ewes.

In Slovakia the problem of environmental mastitis has gradually increased since 2000. First, there were mainly inflammations of the udder caused by *Streptococcus uberis*, *Escherichia coli* and bacteria from the family *Enterobacteriaceae*. Gradually the cases induced by CNS *Pseudomonas spp.*, but also bacteria *Proteus spp.*, and the incidence of mastitis induced by *Arcanobacterium pyogenes* and *Streptococcus dysgalactiae* have increased [4]. Pitkälä, *et al.* [10] reported that a half of the bacteria isolated at environmental mastitis in dairy cows in Finland are the coagulase-negative staphylococci. Monday and Bohach [30] present in their study that CNS are the most prevalent pathogens causing SM in dairy ruminants. Although less pathogenic than *S. aureus*, CNS can also produce persistent SM as well as producing thermostable enterotoxins. Nevertheless, despite the accepted role of these bacteria as major IMI causing pathogens in dairy ruminants, the pathogenicity of the different CNS species varies widely. In our study, CNS, *S. aureus* and streptococci were most common isolated from SM what is generally seen as an increase in the SCC in milk of the infected quarter with positive CMT score. The economic losses are more associated with SM. However the cost of treatment of SM is much low compared to that of CM accounting for 10 - 20 times higher [31].

Subclinical mastitis should be always suspected as one of the primary causes in cases of decreased milk production in dairy herds [11]. In fact, CNS, which are the most common etiological agents of SM, are also frequent inhabitants of the skin of the udder. Most mastitis occurs before the end of lactation (at the beginning of dry period) and also during the rearing lambs [32,33].

Given the variety of factors causing the IMI will be milk production and economic prosperity depend on the expertise and capabilities of the undertaking holding the managers or owners of dairy cows and sheep herds very quickly to implement preventive anti-mastitis methods and technological systems to their own agricultural production. In addition to the breeding of dairy cows and sheep to produce milk, a common aim in marginal regions, which are disadvantage compared to other areas, is to continue in the agricultural activity to maintain or improve the environment, maintain landscape and tourist potential. Without an active agricultural activity in these areas causing the devastation of the country, the environment and the depopulation of the countryside.

Conclusion

In monitored dairy cows and sheep herds situated in the marginal areas on the etiology of mastitis mainly participate bacteria of *Staphylococcus spp.* (mainly CNS and *S. aureus*), and *Streptococcus uberis*. From the point of view of the epidemiological importance, the high contagious bacteria (*S. uberis* and *S. aureus*) that colonise udder, very quickly are contaminated the hands of the milkers or milking machine, is spread from the milking process exclusively. On the other hand, CNS represent a high risk for the formation of new, mainly subclinical infections of environmental origin. The breeders from the monitored dairy herds should be respected the Mastitis Control Program which consist from:

- a) Pre-milking hygiene - udder toilet
- b) After milking - disinfection of all teats
- c) Animals with mastitis must be separated from healthy and individually milked as the last
- d) All acute and subacute cases of mastitis treat according to actually sensitive to antibiotics
- e) Monitoring milk quality and composition of the treated animals after inclusion in the milking process
- f) Animals with chronic mastitis with atrophy of secretory tissue in udder quarters/halves after unsuccessful treatment must be rejected
- g) Unselective treatment of udder with antibiotics, on the start dry period
- h) New dairy animals can be integrated to herd after completely control of health status
- i) Increase the cleaning frequency of stable and changing of litter.

Polyetiological and multifactorial origin of mastitis in dairy ruminants means that the effectiveness of generally applicable Mastitis Control Program in the reduction of environmental mastitis bacteria, in combination with the main contagious pathogens of the mammary gland tends to be limited. Even in marginal conditions should still look for the most efficient systems, appropriate breeds and types of production, using of the rational technological systems and modern farming techniques.

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